EXHIBIT C

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Protocol for Labeling Human Lymphocytes with Anti-IRP-2 Antibodies

Human blood was received (one tube labeled AD sample and the other labeled healthy sample). The Lymphocytes were isolated from the whole blood using PrepaCyte separation medium (BioE). The isolated lymphocytes were washed and resuspended at $1x10^6$ cells/ml. Each sample was distributed to two tubes one for incubation with anti-IRP-2 and the other tube secondary fluorescent label only (FITC). The incubation period was 30 minutes at room temperature (dark), followed by washing twice in phosphate buffered saline that contained 0.05% Tween-20 and 1% bovine serum albumin. The washed cells were then counterstained with propidium iodide (PI) (20 minutes), washed once and spread onto microscope slides. The cells were protected with permafluor mounting medium and glass coverslips and allowed to dry flat in the dark overnight.

The labeled cells on standard microscope slides were scanned on a confocal microscope (BioRad 1024, Life Science Research Hercules, CA). The cells were imaged for green fluorescence (IRP-2, FITC) by excitation with the 488nm line from an argon laser, and the emission viewed through a 496-505nm-band pass filter. The PI stained nuclei were imaged by absorbance using a 550nm line from the argon laser simultaneously and the emission viewed through a 594-620nm band-pass filter. The merged (red/green) images were exported as TIFF files.

The micrograph shows duplicates of the AD patient sample (panel a) and the normal sample (panel b). There is a clear difference between the staining of the peripheral blood cells in the AD patient as compared to the normal. More specifically, the results (in panel a) show that the IRP-2 antibodies (green) specifically stain the IRP-2 in a more diffuse pattern and that there is an increased amount in the AD patient. The control (panel b) shows that the staining is lighter and specifically appears as green dots.